The Effect of Elevated Selenium Intake on Colonic Cellular Growth in Rats

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Abstract

Both selenium and calorie restriction are anticarcinogenic in many tumor models, but the mechanisms of action are unknown. This study compared the effects of elevated selenium (Se) intake and calorie restriction on colonic cellular growth. Female weanling rats were divided into four groups: control, 40% calorie restricted, and 4 or 6 mg Se/l H_2O as selenate. Control rats and rats given Se consumed the control diet ad libitum. Rats in the 40% calorie-restricted group were pair fed 40% less than the total intake of control rats with a diet designed to provide equal nutrients except calories from carbohydrate. After three weeks, rats were injected with [^{3}H]thymidine (1 μCi/g body wt) and killed one hour later. Se at 4 and 6 mg/l H_2O and 40% calorie restriction significantly decreased food intake, weight gain, colon weight, and total colon DNA compared with controls. Total number of cells per crypt was not affected by any treatment, whereas total DNA synthesis was significantly decreased, suggesting that the total number of colonic crypts are reduced by calorie restriction and Se treatment. The rate of cell division was decreased only in rats given 6 mg Se/l H_2O. These results indicate that elevated Se intake and caloric restriction decrease colonic mucosal growth by decreasing growth in general, but only very high intakes of Se affect colonic cell turnover.
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Introduction

Certain epidemiological studies suggest that the incidence of cancer at various sites is inversely associated with selenium (Se) status (1–3). In some experimental animal models, supplemental Se compounds at high levels are effective chemopreventive agents against tumors originating in epithelial tissues such as liver (4), skin (5), mammary tissue (6), and colon (7,8). The mechanism of Se action is unknown, but hypotheses include protection

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against lipid peroxidation, modification of carcinogen metabolism or carcinogen-DNA interactions, and inhibition of DNA or protein synthesis (9).

Evidence indicates that body weight and caloric intake are positively correlated with tumor incidence in rodents (10–13); it has been suggested that decreased body size, resulting in fewer total cells that undergo mitosis and are susceptible to mutation, may be a key factor related to mammary and colon carcinogenesis (14). Growth inhibition also results from exposure to elevated intakes of Se (15). This study, therefore, was designed to compare the effects of elevated Se intake and calorie restriction on cellular growth and cell proliferation in rat colon.

Materials and Methods

Animals

Timed-pregnant Sprague-Dawley dams (Charles River, Wilmington, MA) were received in the laboratory seven days before parturition. Dams were housed in plastic cages with wood shavings used as bedding and were allowed free access to Purina Rat Chow (Product 5001, Ralston Purina, St. Louis, MO) and distilled water during pregnancy and lactation. Temperature was controlled at 24 ± 1°C, and a 12:12-hour light-dark schedule was maintained. One day postpartum, litter sizes were equalized to 12 pups per litter. No rejection of added pups was noted, and lactation proceeded without difficulty. At the time of weaning (21 days postpartum), pups of each litter were rank ordered by sex and body weight and housed individually in wire mesh cages. The pups were randomly assigned to one of four groups: control, calorie restricted, 4 mg Se/l H_2O, or 6 mg Se/l H_2O. Se in the drinking water was in the form of Na_2SeO_4. The levels of Se used in this study were chosen to reflect dose and route used in several experimental carcinogenesis studies (16). Body weight was measured at weaning, weekly until the end of the experiment, and just before killing.

Diets

The diets were based on the formulations of Ruggeri and co-workers (17) as previously described (13). Rats in the control group and rats in the 4 and 6 mg Se/l H_2O groups were fed the control diet ad libitum. Rats in the calorie-restricted group were pair fed 40% less than the nearest weight-matched (immediately postweaning) and sex-matched animal in the control group with a diet providing equal nutrients except calories from carbohydrate. Food spillage was estimated and accounted for daily. Animals were fed daily (late in the afternoon) and were maintained on these dietary regimens for 21 days.

Experimental Procedures

Female rats only were used for the experimental procedures. Although we intended to have seven male and seven female rats in each group, mistakes in ascertaining the sex of the rats at weaning were discovered at the end of the feeding period. Consequently, there were seven female rats each in the control and calorie-restricted groups and five and six female rats each in the 4-mg and 6-mg Se-treated groups, respectively. Rats were injected with [^{3}H]thymidine, and colons were prepared for histology and extraction of DNA, RNA, and protein as previously described (13). The analytical procedures including autoradiography and quantitation of DNA, RNA, and protein were also as described (13).

Statistical Analyses

Data were analyzed by ANOVA and tested with Duncan's multiple-range test (NWA STATPAK, Northwest Analytical, Portland, OR).

Results

Food intake, calorie intake, weight gain, and body length were significantly reduced in calorie-restricted rats and in rats drinking water containing 4 or 6 mg Se/l as compared with controls fed ad libitum (Table 1). At 4 mg Se/l H_2O, rats ate 39% more calories but gained the same amount of weight as calorie-restricted rats did. At 6 mg Se/l H_2O, rats ate 10% more calories but gained 44% less weight than calorie-restricted rats did, resulting in significantly reduced calorie efficiency ratios in both groups of Se-treated rats compared with calorie-restricted and control animals. Body length was significantly reduced in rats given 6 but not 4 mg Se/l H_2O compared with calorie-restricted rats.

Colon weight, but not length, was significantly reduced in calorie-restricted and Se-treated rats compared with controls (Table 2). Total colon DNA, RNA, and protein were also significantly reduced in calorie-restricted and Se-treated rats compared with controls, but cell size (protein-DNA ratio) was not affected by any treatment. There were no significant differences in colon weight or DNA, RNA, or protein content between calorie-restricted and Se-treated rats. Total DNA synthesis (dpm/colon) was significantly reduced in calorie-restricted rats and in both Se-supplemented groups compared with controls. By contrast, the rate of cell division (dpm/mg DNA) was significantly decreased only in the rats given 6 mg Se/l H_2O compared with all other groups.

Results of the histological analyses indicate that the only significant differences appear in the number and percent of labeled cells in the proximal portion of the colon (Table 3). Both groups of Se-treated rats had significantly fewer labeled cells and a lower percentage of labeled cells compared with calorie-restricted and control rats. A similar trend is present in the distal colon, but the results lack statistical significance.

Discussion

The results of this investigation indicate that calorie restriction and elevated Se intakes significantly reduce colonic growth in rats. The number of cells within each crypt (crypt

Table 1. Effect of Elevated Selenium Intake and Calorie Restriction on Food Intake and Body Weight and Length of Rats[a,b]

Group	No. of Animals/ Group	Food Intake, g	Calorie Intake, kcal	Weight Gain, g	Calorie Efficiency Ratio[c]	Final Weight, g	Body Length, cm
Control	7	$248 \pm 25^{*}$	$905 \pm 93^{*}$	$98 \pm 13^{*}$	$0.11 \pm 0.01^{*}$	$131 \pm 15^{*}$	$15.2 \pm 0.5^{*}$
Calorie restricted	7	$158 \pm 20^{\dagger}$	$548 \pm 68^{\dagger}$	$61 \pm 7^{\ddagger}$	$0.11 \pm 0.01^{*}$	$95 \pm 8^{\ddagger}$	$13.7 \pm 0.6^{\ddagger}$
Given 4 mg Se/l H_2O	5	$209 \pm 34^{\ddagger}$	$763 \pm 122^{\ddagger}$	$68 \pm 8^{\ddagger}$	$0.09 \pm 0.01^{\ddagger}$	$101 \pm 10^{\ddagger}$	$14.3 \pm 1.3^{*,\ddagger}$
Given 6 mg Se/l H_2O	6	$166 \pm 38^{\dagger}$	$606 \pm 138^{\dagger}$	$38 \pm 15^{\dagger}$	$0.06 \pm 0.02^{\dagger}$	$72 \pm 15^{\dagger}$	$12.6 \pm 1.1^{\dagger}$

a: Values are means ± SD. Means within a column not sharing common superscripts are significantly different ($p < 0.001$). Data were analyzed by ANOVA and were tested with Duncan's multiple-range test.
b: Total food intake, calorie intake, and weight gain were measured for 3 wks. Final weight and rat length were measured on day of death.
c: Calorie efficiency ratio is calculated as weight gain/calorie intake.

Table 2. Effect of Elevated Selenium Intake and Calorie Restriction on Rat Colon Size, Macromolecule Content, and DNA Synthesis[a]

Group	No. of Animals/ Group	Colon Weight, g	Colon Length, cm	Total Colon DNA, mg	Total Colon RNA, mg	Total Colon Protein, mg	Protein:DNA Ratio	Total DNA Synthesis, dpm/colon $\times 10^{-5}$	Rate of Cell Division, dpm/mg DNA $\times 10^{-5}$
Control	7	$0.77 \pm 0.12^{*}$	$17.2 \pm 1.8^{*}$	$5.3 \pm 0.8^{*}$	$4.2 \pm 0.6^{*}$	$69.1 \pm 10.9^{*}$	$13.0 \pm 1.3^{*}$	$12.1 \pm 2.8^{*}$	$2.27 \pm 0.73^{*}$
Calorie restricted	7	$0.63 \pm 0.06^{\dagger}$	$15.4 \pm 1.4^{*}$	$3.9 \pm 0.9^{\dagger}$	$3.1 \pm 0.9^{\dagger,\ddagger}$	$46.7 \pm 14.5^{\dagger}$	$11.8 \pm 1.5^{*}$	$8.4 \pm 1.7^{\dagger}$	$2.21 \pm 0.34^{*}$
Given 4 mg Se/l H_2O	5	$0.60 \pm 0.06^{\dagger}$	$15.8 \pm 1.9^{*}$	$4.1 \pm 0.5^{\dagger}$	$3.4 \pm 0.4^{\dagger}$	$52.0 \pm 10.3^{\dagger}$	$12.5 \pm 1.3^{*}$	$9.7 \pm 1.8^{*,\dagger}$	$2.34 \pm 0.23^{*}$
Given 6 mg Se/l H_2O	6	$0.52 \pm 0.07^{\dagger}$	$15.1 \pm 1.3^{*}$	$3.5 \pm 0.3^{\dagger}$	$2.6 \pm 0.4^{\ddagger}$	$42.2 \pm 6.0^{\dagger}$	$11.9 \pm 1.1^{*}$	$5.4 \pm 1.4^{\ddagger}$	$1.53 \pm 0.33^{\dagger}$
P Value[b]		<0.001	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.05

a: Values are means ± SD. Means within a column not sharing common superscripts are significantly different as indicated. Data were analyzed by ANOVA and were tested with Duncan's multiple-range test.

b: NS, not significantly different.

Table 3. Effect of Elevated Selenium Intake and Calorie Restriction on Colon Histology in Rats[a]

	Control (n = 7)	Calorie Restricted (n = 7)	Given 4 mg Se/l H_2O (n = 5)	Given 6 mg Se/l H_2O (n = 5)[b]
Crypt height, no. cells/crypt				
Proximal	30.6 ± 1.4	30.2 ± 1.8	28.9 ± 2.0	29.7 ± 2.0
Medial	40.6 ± 0.9	38.9 ± 2.8	39.0 ± 3.6	40.1 ± 3.2
Distal	37.1 ± 2.7	37.5 ± 2.2	37.8 ± 3.4	36.0 ± 3.3
No. of labeled cells/ crypt column				
Proximal	3.3 ± 0.7*	2.9 ± 0.6*	1.7 ± 0.9†	2.0 ± 0.7†
Medial	2.2 ± 0.8	2.2 ± 0.7	2.0 ± 0.3	2.2 ± 0.9
Distal	2.6 ± 0.7	2.5 ± 1.3	2.1 ± 0.9	1.5 ± 0.9
% Labeled cells				
Proximal	10.8 ± 2.2*	9.4 ± 1.8*,†	5.6 ± 2.9‡	6.7 ± 2.5†,‡
Medial	5.6 ± 2.0	5.6 ± 1.5	5.3 ± 0.9	5.5 ± 2.6
Distal	7.0 ± 2.1	6.8 ±3.5	5.5 ± 2.0	5.3 ± 2.3
Highest labeled cell				
Proximal	16.7 ± 3.6	17.3 ± 5.5	13.6 ± 2.4	15.4 ± 3.8
Medial	26.6 ± 3.0	22.9 ± 2.9	23.2 ± 3.6	24.8 ± 5.6
Distal	19.1 ± 3.3	18.6 ± 4.2	16.8 ± 4.0	16.6 ± 1.8

a: Values are means ± SD. Means within a row not sharing common superscripts are significantly different ($p < 0.001$). Data were analyzed by ANOVA and were tested with Duncan's multiple-range test.
b: One animal was lost in this group.

height) was not affected by either calorie restriction or Se treatment, whereas the total number of cells (total colon DNA) and, therefore, the total number of dividing cells (dpm/colon) were decreased in all experimental groups. This suggests that the total number of crypts is reduced by calorie restriction and Se treatment. In rats given 6 mg Se/l H_2O, cell proliferation (dpm/mg DNA) was also significantly reduced; the proximal colon was the site of this reduction.

Elevated Se intakes led to a reduction in weight gain and food consumption but, more importantly, to a decrease in food utilization. Se-treated rats gained less weight per calorie of food consumed than did both control and calorie-restricted rats. Although other researchers have reported decreased weight gain and food intake as a result of treatment with high levels of Se (15,18), the results of this study suggest that elevated Se intakes disrupt efficiency of energy utilization. Both the calorie-restricted rats and the animals receiving 4 mg Se/l H_2O increased their weight and length at the same rate although the animals treated with Se consumed 39% more calories. The reduction in colon weight, length, and total cell number was similar in both calorie-restricted rats and animals receiving 4 mg Se/l H_2O. In both groups the rate of DNA synthesis (dpm/mg DNA) was the same as in the control animals. By contrast, the animals receiving 6 mg Se/l H_2O showed significantly more growth retardation (reduced weight gain and body length) than did either of the other two experimental groups even though they were consuming the same number of calories as the calorie-restricted group did. In addition to the reduced number of cells seen in the other two experimental groups, rats given 6 mg Se/l H_2O also showed a reduced rate of DNA synthesis.

Thus, all of the biochemical data can be explained by the effect of growth retardation on cellular growth and proliferation rate of the colon. Moderate growth retardation produced either by 40% calorie restriction or by ingestion of 4 mg Se/l H_2O results in a reduced cellular growth in the colon, without any effect on the rate of cell division. More marked growth retardation produced by ingestion of 6 mg Se/l H_2O not only results in reduced cellular growth of the colon but also in a reduced rate of cell division. It is possible that the primary effect of Se excess in these experiments is to reduce the number of calories available for growth by inducing a reduction in both caloric intake and food utilization.

Changes in colon histology were seen only as a result of Se treatment and only in the proximal segment of the colon. These results agree with those of Tempero and colleagues (19), who were unable to report an effect of Se on colonic labeling indices in distal colon. By analyzing separate segments of the colon in this investigation, we are able to report that changes do occur but that they are restricted to the proximal segment. The decrease in number and percentage of labeled cells in the proximal colon are also of interest in light of the results of Banner and others (20), who reported that Se uptake is enhanced in the proximal colon compared with the distal colon, effects that parallel the antitumorigenic action of the element. Because the changes in colonic histology are seen in both Se-treated groups, they do not correlate with either food intake or amount of growth failure. The lack of a correlation suggests a specific effect of Se over and above its effect on caloric utilization.

Thus, the results of this study suggest that Se excess may affect colonic cellular growth and proliferation by two independent mechanisms. The first is by making fewer calories available for colonic cellular growth. The second is by direct inhibition of cell division primarily within the crypts of the proximal colon. Further studies in the form of pair-feeding trials are necessary to separate the effects of elevated Se intakes from the effects of decreased food availability on colonic growth.

Acknowledgments and Notes

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